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# Determination of Alkaline Phosphatase Expression in Endodermal Cell Lineages of an Ascidian Embryo

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**Abstract.** *Ciona intestinalis* embryos develop a strong histochemical localization of alkaline phosphatase activity in their known endodermal tissues. Such tissues arise solely from the four vegetal blastomeres at the 8-cell stage and six vegetal blastomeres at the 16-cell stage; these vegetal cells inherit an endodermal lineage cytoplasm. Pairs of blastomeres from the bilaterally symmetrical 8- and 16-cell stages were isolated and reared as partial embryos. Only those partial embryos derived from endoderm-containing lineages developed a histochemically localized alkaline phosphatase activity. From the results of such restricted developmental autonomy (self-differentiation), one can deduce that this enzymic expression of endodermal fate could be specified by events of cytoplasmic segregation that occur during the early cleavages. This conclusion offers additional support to the theory that specification of cell fate in ascidian embryos involves an early differential segregation of histodetermining egg cytoplasmic materials.

## Introduction

Elaboration and refinement of cell lineages and the construction of fate maps for certain widely studied animal embryos continue to be important contemporary parts of experimental embryology. Fate maps produced by marking early blastomeres of the embryo indicate what actually happens to each cell or region as it develops, and are an essential component of attempts to interpret the causal relations concerned with eventual regional specializations in the embryo. Yet fate maps do not indicate when, during the succession of cleavages, cells become irreversibly committed to the pathways of differentiation they later exhibit during histodifferentiation. Our one, and still only, radical criterion for discovering whether the prospective fate or "determination" of a blastomere has already been settled during early cleavages is that, when the blastomere is isolated from its usual cellular environment and associations, some or all of its progeny cells then differentiate only in the fixed or limited directions predicted by a fate map (Lillie, 1929). The underlying hypothesis is the presumption that eventual cell fate is determined by certain localized cytoplasmic agents that become differentially segregated only into certain cells (Whittaker, 1987). This paper is such an investigation of specification in the endodermal cell lineages of *Ciona intestinalis.* These embryonic cells eventually become the branchial and digestive tissues of the postmetamorphic juvenile and adult.

The first accurate and extensive cell lineages for ascidian embryos were described by Conklin (1905). Endodermal lineages are segregated at third cleavage (8-cell stage) to the vegetal four cells of the embryo and thereafter become progressively more restricted to certain vegetal regions of the dividing embryo. Based on general characteristics of cytoplasmic morphology and staining (Conklin, 1905, 1911), endodermal lineages appear to inherit a particular kind of cytoplasm. Conklin's original designations of the endodermal lineages have been confirmed recently in studies using injected horseradish peroxidase (HRP) as a cell lineage marker (Nishida and Satoh, 1983, 1985; Nishida, 1987).

Quite early in embryogenesis, beginning at the neurula stage, endodermal tissues develop localizations of an essentially histotypic alkaline phosphatase; other larval tissues lack any significant amount of it (Minganti, 1954a; Whittaker, 1977). In certain species, development of this enzyme is a simple and unequivocal indication of endodermal differentiation. Ascidian embryos, which are di-

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vision-arrested at various early cleavage stages with cytochalasin B, eventually express alkaline phosphatase only in those cells that are known to be of endodermal lineage (Whittaker, 1977; Satoh, 1982). This suggests further that endodermal expression follows a pattern of cytoplasmic localizations. However, in such cleavage-arrested embryos, the enzyme-developing cells are not separated from the influence of their surrounding cells. The present study examines alkaline phosphatase development in partial embryos originating from blastomere pairs of the bilaterally symmetrical embryo isolated from early cleavage stages. Eventual enzyme expression exclusively follows the known endodermal lineages. Such developmental autonomy is likewise consistent with the hypothesis of a differentially segregated cytoplasmic determinant.

## Materials and Methods

### *Organisms*

Adult *Ciona intestinalis* (L.) were collected near Woods Hole, Massachusetts, and maintained on sea tables with continuously flowing seawater and under constant light. *Phallusia mammillata* (Cuvier) was obtained from two sources: from the Gulf of Palermo in Sicily by courtesy of the Institute of Zoology at the University of Palermo, and from the coast of Brittany at Roscoff (France) through the kindness of Dr. Lionel Jaffe. Eggs from two or more animals were removed surgically from the oviducts and fertilized with diluted sperm obtained from the sperm ducts of other adults. Embryos were dechorionated manually before first cleavage with sharpened steel needles and cultured at 18 ± 0.1°C in sterile Millipore-filtered (0.2 μm porosity) seawater containing 0.1 m*M* EDTA (Crowther and Whittaker, 1983). Under these conditions, larvae became fully developed by 18 h from fertilization.

### *Blastomere isolations*

Dechorionated embryos of the bilaterally symmetrical 8-cell stage of *Ciona* were the starting point for isolations of various cell pairs at the 8- and 16-cell stages. Blastomeres were separated with agar-coated glass filament needles. Partial embryos from various isolations were then reared in agar-coated Syracuse watch glasses for periods of development up to 20 h before they were processed histochemically for an alkaline phosphatase reaction.

### *Cleavage inhibition*

Exposure to cytochalasin B (Aldrich) (2 μg/ml) prevented cell division in embryos (Crowther and Whittaker, 1983).

### *Alkaline phosphatase histochemistry*

The 80% cold ethanol fixative used previously with chorionated ascidian embryos (Whittaker, 1977) gave satisfactory but often variable results when applied to dechorionated partial embryos. This variability was eliminated by employing the glutaraldehyde-formaldehyde fixative devised by Karnovsky (1965) for electron microscopic histochemistry; it was used here at 0.5% each, half the suggested aldehyde concentrations. One-hour fixation (5°C) and 20-min wash in the recommended sucrose-containing cacodylate buffer (Karnovsky, 1965) produced excellent and reproducible results with the subsequent alkaline phosphatase reaction. Background staining was insignificant under these conditions.

Fixed whole or partial dechorionated *Ciona* embryos were reacted for 24 h (at 18°C) in standard Gomori medium with β-glycerophosphate as substrate and the calcium phosphate product afterwards visualized by a silver reduction technique (Pearse, 1972). This results in a stable brown deposit of reduced silver at the sites of alkaline phosphatase activity. The stained specimens were dehydrated in ethanol, cleared in xylene, and mounted in dammar resin.

Some staining for alkaline phosphatase activity on larvae and dechorionated whole and partial embryos was done with a tetrazolium method using bromochloroindoxyl phosphate (BCIP) as substrate (McGady, 1970, and as described by Whittaker and Meedel, 1989). Both fixation methods were used with this procedure; similar results were obtained with each.

### *Acetylcholinesterase histochemistry*

Assays were done on dechorionated whole and partial embryos after using the same aldehyde fixative described above for alkaline phosphatase. The cholinesterase method of Karnovsky and Roots (1964) was applied as described by Meedel and Whittaker (1984).

## Results

### *Endodermal cell fates predicted by cell lineage studies*

The most accurate cell lineage relationships for potential endodermal expression have been obtained from cell marking studies with injected HRP (Nishida and Satoh, 1983, 1985; Nishida, 1987). Figure 1 is a lineage diagram of the cell contributions up to the 16-cell stage: at the 8-cell stage the four vegetal blastomeres (the bilateral pairs of A4.1 and B4.1) contain endodermal lineages; at the 16-cell stage only six of the eight vegetal cells contain endodermal lineages. The sensitivity of the HRP detection method has also permitted an identification of the lineage origins of certain smaller structures of the embryo designated in Figure 1 as secondary. Figure 2 depicts in

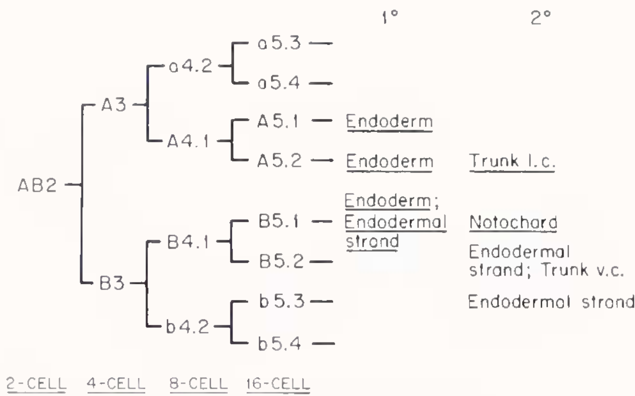



**Figure 1.** Fate map of endodermal cell lineages in ascidian embryos up to the 16-cell stage, according to Conklin (1905), Ortolani (1954), and Nishida and Satoh (1983, 1985). Cells from one-half of the bilaterally symmetrical embryo are indicated. Primary (1°) and secondary (2°) tissues which ultimately develop alkaline phosphatase are underlined. Nomenclature is that of Conklin (1905).

a middle tailbud stage (11 h) embryo the various "endodermal" regions actually observed by such studies and their respective origins from the bilaterally symmetrical pairs of blastomeres at the 16-cell stage (identified in Fig. 1).

The regions shaded with diagonal lines in the head part of the embryo diagram (Fig. 2) are the locations of the cells that give rise to the endodermal organs of the postmetamorphic juvenile: a branchial basket and the digestive system. These are the fates of the primary endodermal cells in Figure 1. The tail is a strictly larval structure and its tissues, including the endodermal strand (an extension of the main endodermal mass running mid-ventral to the notochord), and the notochord itself are destroyed at the time of larval metamorphosis. Other tissue areas of the embryo-larva (muscle, neural, and epidermal) are not shown.

Questions about the minor endodermal structures identified in Figures 1 and 2 arise from the cell lineage studies. "Trunk lateral cells" (TLC), which occur in two superficial dorsal wing-like accumulations on either side of the mid-tailbud embryo, are undefined in their fate yet still share a major endodermal lineage as late as the 32-cell stage (Nishida and Satoh, 1985); at the 64-cell stage they separate from endoderm as a separate lineage (Nishida, 1987). Two small circular patches of cells, which I have called "trunk ventral cells" (TVC), occur along either side of the embryo ventral midline at the base of the tail. These have been classified as endodermal by Nishida (1987) because of their location in an endodermal region, but they originate after the 128-cell stage from a lineage (B5.2) that is entirely mesodermal from the 16-cell stage onwards. Finally, two short distal segments of the endodermal strand originate from cells (B5.2 and b5.3 cell pairs) which, after the 16-cell stage, are not otherwise endodermal lineages.

### *Alkaline phosphatase expression in tissues of endodermal lineage*

Because differential alkaline phosphatase expression has been regarded as a histotypic indicator of early differentiation in endodermal tissues of some ascidian species (Minganti, 1954a, and others), occurrence of enzyme in the so-called secondary larval tissues (Fig. 2) would be a confirmation of their endodermal specification. The Gomori and BCIP methods of enzyme detection have been applied to 11-h (middle-tailbud stage) *Ciona* embryos and also to embryos cleavage-arrested in cytochalasin B at 11 h and fixed for reaction at 20–28 h postfertilization. Cleavage-arresting dechorionated 11-h embryos has the interesting effect of causing an earlier and more concentrated development of enzyme in cells that might ordinarily divide further. Alkaline phosphatase does not usually occur strongly in endoderm-derived tail tissues until 6–8 h after hatching. Figure 3 shows the BCIP staining of such a cleavage-arrested larva. In previous lineage studies, the middle tailbud stage was used as the reference stage for tissue locations (Nishida, 1987; Nishida and Satoh, 1983, 1985). Cleavage-arrested 11-h embryos (Fig. 3) enable us to make a direct comparison to those results (Fig. 2).

Only tissues arising from lineages that share a primary endodermal lineage until after the 16-cell stage are seen to develop alkaline phosphatase. This includes the eight cells at the tip of the notochord, which have such an endodermal lineage origin but are not structurally or functionally endodermal cells; their lineages first separate from endoderm at the 32-cell stage. The two short terminal segments, which are structurally a part of the endodermal strand, but which do not share endodermal lineages after the earliest cleavages, do not produce alkaline phosphatase.

During the first 30 min of BCIP staining, one can see the TLC reacting strongly against the initially lighter staining of the underlying other endodermal cells. The staining time required to reveal the endodermal strand clearly (2–3 h) soon results in sufficient reaction product in the head region to obscure the TLC. During initial staining, or later, one can not see any differential alkaline phosphatase stain in the TVC region. In normal 11-h embryos, one finds the same differential staining with BCIP in the TLC, but at that time enzyme has not yet developed in the endodermal strand or notochordal tip cells, as it has in the cleavage-arrested embryos reacted after "hatching." Given their sharing of an endodermal lineage, and their expression of alkaline phosphatase, it seems unlikely that the TLC would be the precursors of

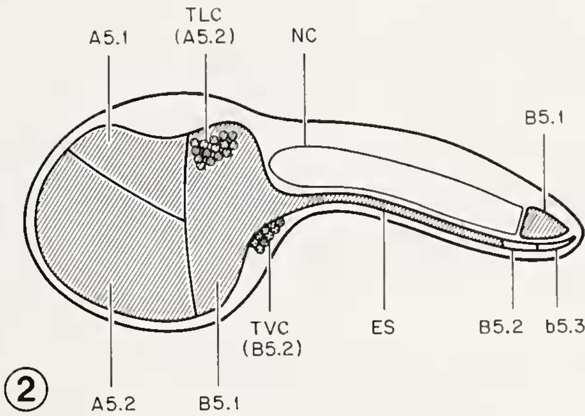


**Figure 2.** Diagram of the middle tailbud stage (11 h) ascidian embryo showing the endoderm and possibly endodermally related tissues identified from lineage tracing studies (Nishida and Satoh, 1983, 1985; Nishida, 1987). Lineage origins at the 16-cell stage are given for each defined tissue region. Those tissues that eventually express alkaline phosphatase, including the trunk lateral cells (TLC), the proximal endodermal strand (ES), and the distalmost cells of the notochord (NC), are indicated with fine diagonal lines. The (stippled) trunk ventral cells (TVC) develop acetylcholinesterase but not alkaline phosphatase.

**Figure 3.** Dechorionated 11-h (middle tailbud) *Ciona intestinalis* embryo cleavage-arrested at 11 h with cytochalasin B and reacted at 28 h (after fixation) for alkaline phosphatase with the BCIP reagent. Incubation time is 2 h. Bar = 50 $\mu$m.

juvenile blood cells (a presumably mesodermal derivative), as suggested by Nishide *et al.* (1989).

The Gomori stain is much less sensitive than the BCIP stain. It does not reveal any enzyme activity in the endodermal strand, but shows clearly the TLC staining differentially in the head region (not shown). The two bilateral TVC regions stain significantly for acetylcholinesterase, a characteristic expression of differentiating muscle and mesenchyme tissue regions (Meedel and Whittaker, 1979). This TVC staining (not shown) occurs in normal 11-h embryos as well as 11-h cleavage-arrested embryos. By their location, these apparently mesodermal tissues may be the primordia of the juvenile heart. Although the inner distal segment of the endodermal strand originates (at the 64-cell stage) from what is otherwise a mesodermal lineage (B5.2), these tissues do not develop an acetylcholinesterase. They thereby differ from the notochordal tip cells by not expressing a vestige of their secondary origin.

### *Design of cell isolation experiments*

At the 8-cell stage, third cleavage divides the bilaterally symmetrical embryo equatorially across the animal-vegetal axis into a vegetal quartet of cells containing the endodermal lineages and an animal quartet that has no endodermal fate (Fig. 4A). Ortolani (1954) used adhering carbon particles to mark the surface areas of the four vegetal blastomeres which eventually appear in endodermal tissues. Under conditions of appropriate lighting (see below) one can actually observe the endodermal regions indicated in Figure 4A by the diagonal lines, to contain much more yolky material than the other part of the cell, which remains noticeably clearer; these yolky areas have relatively sharp edges. This confirms Conklin's (1905) observations as well. In this investigation, various blastomere pairs have been isolated microsurgically at the 8- and 16-cell stages (Figs. 4B, 4C).

### *Separation of lineage blastomeres*

The *Ciona* 8-cell stage has a very characteristic pattern in lateral aspect (Figs. 4A, 5) by which the four cell pairs can be identified. Often the pair of polar bodies can be seen resting on the a4.2 cells at the animal pole as diagrammed in Figure 4A, but the shapes and apparent sizes of the cells are most diagnostic of their lineage. In doing the isolations, overhead lighting (from a quartz halogen fiber optic lamp) is preferred, with a substage mirror set to reflect back some of this illumination. With properly balanced direct/indirect lighting, the vegetal cell pairs (A4.1 and B4.1), which contain more yolk, appear slightly darker than the others. This facilitates recognition of the A4.1 cells, which thereby appear darkest. The B4.1 cells are somewhat flattened in the animal-vegetal direction and seem to be slightly larger in lateral view than the other pairs of cells.

Animal and vegetal quartets (half-embryos) were isolated by separating the sets as shown in Figure 4B. The cell size and pattern of arrangement in the animal and vegetal quartets (Figs. 6, 7) further simplifies identification of the various cell pairs at the "8-cell stage" in obtaining quarter-embryos (Fig. 4C). Similarly, when the A4.1 and B4.1 blastomere pairs divide again at the "16-cell" stage, there were characteristic sizes and patterns of cells (Figs. 8, 9), which enabled one to isolate the correct lineage pairs.

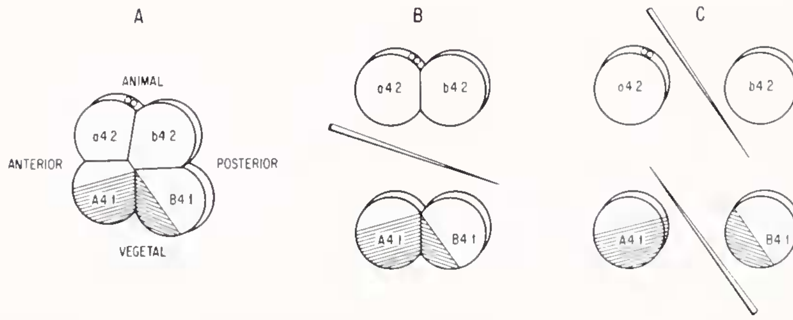
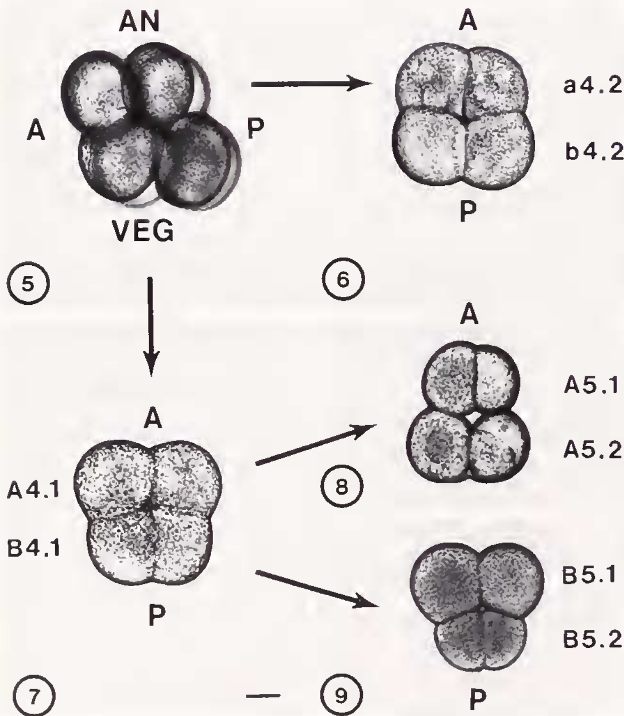



**Figure 4.** Diagrams of the surgical operations involved in isolating blastomeres from the 8-cell stage (A). Isolation of animal and vegetal quartets (B) and quarter-embryos (C). The endodermal territories as mapped by Ortolani (1954) are indicated by diagonal lines.

Size and pattern of the cells can be learned initially from Conklin's (1905) diagrams, which are exceptionally accurate. One can also observe the shapes, sizes, and positions of cells (*in situ*) when isolated half-embryos divide again in culture. Partial larvae resulting from given blastomere pairs have very distinctive morphologic features by which the accuracy of one's initial selection is easily confirmed.




**Figures 5–9.** *Ciona intestinalis* embryo and isolated blastomeres photographed after brief fixation in the Karnovsky (1965) fixative. Figure 5: 8-cell stage in lateral view as in Figure 4A. Figure 6: Animal quartet of cells from 8-cell stage. Figure 7: Vegetal quartet of cells from the 8-cell stage. Figure 8: Isolated A4.1 cell pair after the next division. Figure 9: Isolated B4.1 cell pair after the next division. The embryo orientation letters are AN (animal), VEG (vegetal), A (anterior), and P (posterior). All magnifications are the same; bar in Figure 7 = 50 $\mu$m.

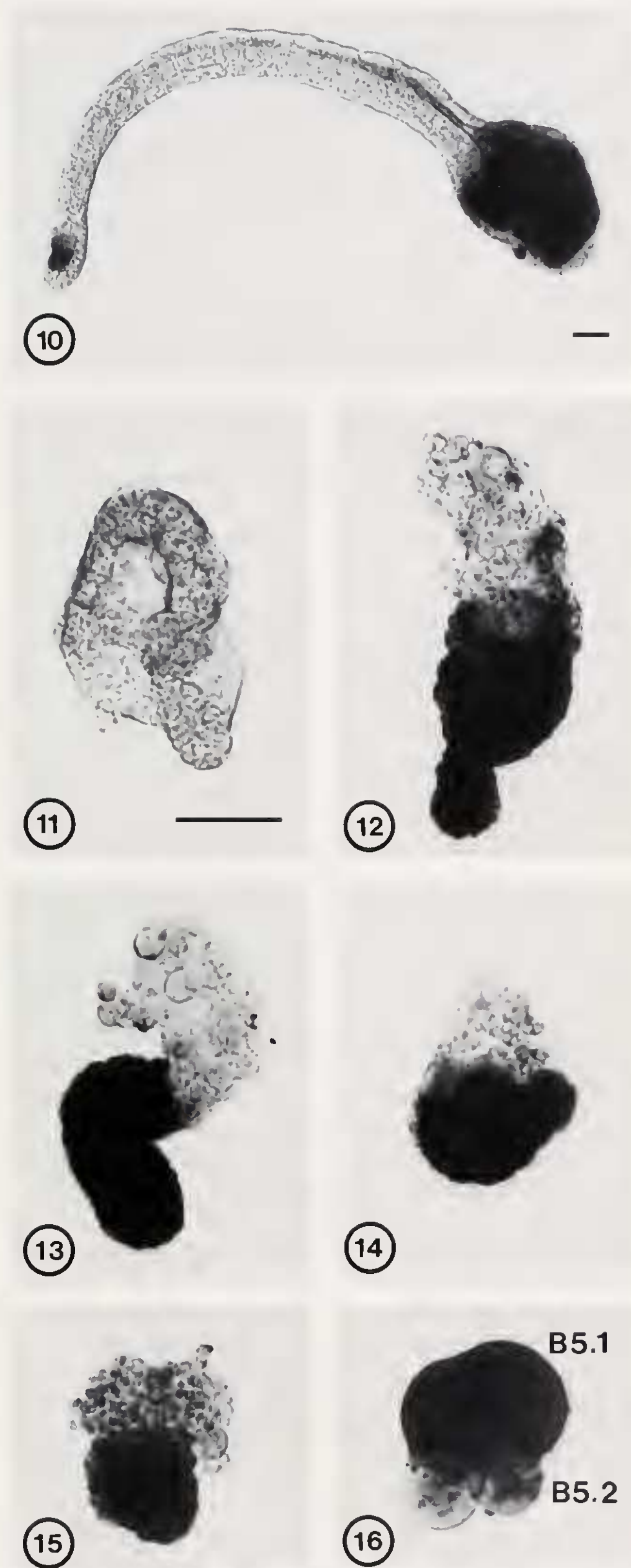
### *Alkaline phosphatase development in partial embryos*

Only partial embryos originating from blastomeres known to contain endodermal lineages (Fig. 1) developed groups of cells containing alkaline phosphatase in the resulting partial larvae (Table I). In larvae developing from dechorionated whole embryos, there was always enzyme staining in the endodermal mass of the head region (Figs. 3, 10). Normal specimens were included as positive controls in each of the various enzyme incubations (Gomori method) with partial embryos. Some larvae show staining in the endodermal strand along the tail and in the few notochordal cells at the tip of the tail; both are evident in Figure 10. Staining of the endodermal strand and notochord did not usually occur until 6–8 h after hatching of controls (18 h), and appeared progressively stronger with time.

**Table I**

*Development of histochemically localized alkaline phosphatase in isolated partial embryos of* Ciona intestinalis

| Blastomeres isolated | Number of experiments | Embryos examined (positive reaction/total) |
|---|---|---|
| *8-cell stage* | | |
| animal quartet | 7 | 0/155 |
| a4.2 pair | 4 | 0/45 |
| b4.2 pair | 4 | 0/45 |
| vegetal quartet | 5 | 115/115 |
| A4.1 pair | 6 | 73/73 |
| B4.1 pair | 6 | 107/107 |
| *16-cell stage* | | |
| A5.1 pair | 2 | 42/48 |
| A5.2 pair | 2 | 52/56 |
| B5.1 pair | 3 | 60/62 |
| B5.2 pair | 3 | 1/56 |




**Figures 10–16.** *Ciona intestinalis* embryos after 18–20 h of development, reacted for alkaline phosphatase (Gomori). Bar = 50 μm. Figure 10: Larva from dechorionated whole embryo. Figure 11: Animal half-embryo. Figure 12: Vegetal half-embryo. Figure 13:A4.1 quarter-embryo. Figure 14: B4.1 quarter-embryo. Figure 15: B5.1 eighth-embryo. Figure 16: Divided B4.1 embryo (as in Fig. 9), cleavage-arrested in cytochalasin B, and showing the B5.1 and B5.2 cell pairs.

The experimental results presented here were done with isolated blastomeres from embryos with completely normal cleavage patterns. In association with the experiments of Table I, 5 series containing a total of 162 whole dechorionated embryos selected for normal cleavage patterns were reared to "hatching" time. Ninety percent of these embryos developed to fully formed "normal" larvae. In most respects, *Ciona* larvae originating from dechorionated early embryos are normal, but they lack a properly formed tail fin of the translucent test covering (as previously noted with another species by Cloney and Cavey, 1982).

No animal half-embryos developed enzyme (Fig. 11), nor did any quarter-embryos (a4.2 or b4.2) derived from the animal quartet. Vegetal half-embryos always produced enzyme (Fig. 12), as did the A4.1 and B4.1 quarter-embryos (Figs. 13 and 14) prepared from the vegetal half of the 8-cell stage. Partial embryos developing alkaline phosphatase invariably contained tissue staining over a large area of the resulting partial larva (Figs. 12–15). Three experimental "control" series were done in which animal and vegetal quartets were isolated and cultured only until 4 h after fertilization before they were fixed and reacted. These contained no localized enzyme. Ordinarily, experimental and control embryos were cultured for 18–20 h.

Partial embryos from A4.1 and B4.1 vegetal cells prepared after the next cleavage (the "16-cell" stage) also showed alkaline phosphatase development correlated with the segregation of endodermal lineages. Most of the A5.1 and A5.2 ⅛th-embryos produced a mass of enzyme-containing cells. A few embryos did not react. Almost all of the B5.1 ⅛th-embryos developed enzyme (Fig. 15), and essentially none of the B5.2 embryos (Table I). One B5.2 embryo (out of 56) developed some alkaline phosphatase. Because microsurgical preparation of partial embryos from 16-cell stage blastomere pairs necessarily entailed some sequential slight bruising of the cells, the one B5.2 embryo that produced alkaline phosphatase might reasonably have resulted from a missegregation of cytoplasm caused by such trauma.

A striking further illustration of the correlation between alkaline phosphatase development and endodermal lineage segregation occurs with a cleavage-arrested partial embryo. B4.1 pairs were isolated (Fig. 4C) and, before being treated with cytochalasin B, were permitted to undergo an additional cleavage creating an embryo with B5.1 and B5.2 daughter pairs (Fig. 9). Almost all of

these embryos (17 out of 18) had staining in both B5.1 cells and none had staining in the B5.2 cells (Fig. 16). This experiment complements work in a previously published study on expression in cleavage-arrested *Ciona* embryos (Whittaker, 1977).

When the more sensitive BCIP staining method was used to localize alkaline phosphatase in partial embryos, the same strict lineage expressions could be demonstrated as shown above with the Gomori method. However, separating blastomeres before complete closure of the cytoplasmic bridges between daughter cells sometimes caused transfer of very small amounts of cytoplasm, which resulted subsequently in tiny regions of enzyme expression. These expressions could not be detected with the less-sensitive Gomori technique. Such transfers were avoided by a change in isolation techniques. The results will be described in detail elsewhere in another context.

*Alkaline phosphatase in* Phallusia *embryos*

When hatched *Phallusia* larvae were reacted for alkaline phosphatase uniformly dark Gomori and BCIP staining reaction products occurred in all the tissues, with no indication of localized staining. Similarly, when dechorionated eggs and embryos of early cleavage stages were reacted for enzyme, a same dark reaction product was also found throughout the whole. With each method, this screen of general staining obscured any possibility of seeing a localized specific staining that might otherwise develop in endodermal tissues. These stainings appear to result from the activity of a universally distributed phosphatase enzyme already present in the egg.

Unfortunately, Minganti (1954a) failed to note this staining of *Phallusia* larvae in his survey of several ascidian species. His further observation that partial embryos of *Phallusia* originating from isolated animal and vegetal quartets of the 8-cell stage both have staining (Minganti, 1954b) proves to be correct, but indicative only of enzyme already present and not of new alkaline phosphatase formation in the animal half-embryo during development.

## Discussion

The classic study by Reverberi and Minganti (1946) on the fate of blastomere pairs isolated at the 8-cell stage showed that anterior and posterior vegetal pairs (A4.1 and B4.1) give rise to quarter-embryos containing some general histological features of organization resembling early gut tissues. Quarter-embryos arising from the animal blastomere pairs (a4.2 and b4.2) did not have this organization, but unfortunately such histologic characters lack the discrimination and sensitivity for evaluating minor expressions of endodermal differentiation. Except for a predominance of yolk granules in cells of endodermal lineages and some other lineages (*e.g.*, notochordal) derived from the vegetal half of the egg (Mancuso and Dolcemascolo, 1979), there are no simple cytospecific features of endodermal differentiation even at the ultrastructural level. However, a strong alkaline phosphatase development proves to be a simple, sensitive, and essentially histotypic indicator of an early endodermal differentiation, at least in some species. An elevated alkaline phosphatase appears to be a universal constituent of the digestive systems of animals (McComb *et al.*, 1979). As noted in the results, there are ascidians (*Phallusia*) in which any possible differential development of enzyme is obscured by a uniformly distributed strong alkaline phosphatase activity present throughout development, and originating in the egg before development begins.

Results of the present blastomere isolation study indicate that partial embryos derived from "endodermal" blastomeres isolated at 8- and 16-cell stages self-differentiated extensive patches of cells containing high levels of alkaline phosphatase. Embryos obtained from the nonendodermal lineages did not produce alkaline phosphatase, at least at any visual level of differential histochemical staining. These findings are in agreement with the fates indicated by previous lineage studies and with expressions of enzyme observed in cleavage-arrested embryos (Whittaker, 1977, and Fig. 16). A restriction of fate occurs, therefore, in parallel with the lineage. The endodermal lineage map is apparently also a fate map.

The theory behind the early specification of cell fate in ascidian embryos and other so-called mosaically developing organisms is the likelihood of differential segregation of specific egg cytoplasmic materials (Lillie, 1929). Ooplasmic rearrangements that occur immediately after fertilization create visible and presumably chemically distinct regional differences in zygote cytoplasm; these regions become segregated into certain cell lineages as the germ divides (Conklin, 1905, 1911). Cell fate is then ordered by agents or substances (determinants) within these regions of cytoplasmic difference. The present results are consistent with this theory. Some credence can be attached to the theory because muscle lineage fate in ascidians appears to be transferrable to other cells along with myoplasmic cytoplasm (Whittaker, 1987).

One might conclude that endodermal alkaline phosphatase expression is regulated by segregation of an egg cytoplasmic determinant into the major (functional) tissues. Because of their early inclusion in an endodermal lineage, the eight distal notochordal cells that express alkaline phosphatase have probably inherited determinant by segregation. The two distalmost short segments of the endodermal strand are actually not derived from immediate endodermal lineages, do not express enzyme, and would seemingly not have received any determinant.

The unresolved nature of egg cytoplasmic determinants remains an important issue in embryology (Davidson, 1990). In certain cases these agents seem to be masked maternal messenger RNAs for some of the proteins involved in a later developmental change. There is indirect evidence from experiments with actinomycin D and other inhibitors of RNA synthesis that the ascidian alkaline phosphatase determinant could be such a preformed maternal mRNA (Whittaker, 1977; Bates and Jeffery, 1987).

Bates and Jeffery (1987) have observed by histochemistry that "activated" but nonnucleate zygote fragments do not elaborate the endodermal alkaline phosphatase after time. Their results were confirmed by more sensitive quantitative measurements on similar material (Whittaker and Meedel, 1989). Also, aphidicolin, an inhibitor of DNA synthesis, has a time-window effect on *Ciona* alkaline phosphatase development (Satoh, 1982). While such findings do not establish the necessity of a gene transcription, they indicate a possible involvement of nuclear replication events in releasing the expression of alkaline phosphatase. Nuclear division might be related to a mechanism for processing an inactive mRNA.

One possible mRNA processing mechanism to consider is translational activation by polyadenylation of a dormant maternal mRNA. There are some examples in the recent literature of translational activation of dormant mRNAs being accompanied by elongation of their 3′ poly(A) tails (see McGrew *et al.*, 1989). Huarte *et al.* (1987) describe an mRNA for mouse oocyte tissue plasminogen activator that accumulates in the cytoplasm during oocyte growth; translational activation of this mRNA occurs at meiosis and is accompanied by increased 3′-polyadenylation. Actual meiotic changes may be necessary since the mixing of cytoplasm and nucleoplasm at germinal vesicle breakdown is not sufficient to initiate the processing. This meiosis-initiated polyadenylation is insensitive to inhibition of RNA synthesis.

Further speculation about the nature of an alkaline phosphatase determinant would be aided by more direct evidence that there is a differentially segregated egg cytoplasmic factor, and by some information about the conditions under which it functions. The present investigation has established a biological background within which to pursue such questions. A next paper will present evidence that moving endodermal lineage cytoplasm to nonendodermal lineages results in the acquisition of alkaline phosphatase expression.

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